A DISSERTATION FOR THE DEGREE OF MASTER OF SCIENCE

Physiologic and Molecular Basis of Dry Land Adaptability in *Echinochloa* Species

BY

Hyejin Yu

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MAJOR IN CROP SCIENCE AND BIOTECHNOLOGY

DEPARTMENT OF PLANT SCIENCE

THE GRADUATE SCHOOL OF SEOUL NATIONAL UNIVERSITY

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UNDER THE DIRECTION OF PROF. DO-SOON KIM
SUBMITTED TO THE FACULTY OF THE GRADUATE SCHOOL
OF SEOUL NATIONAL UNIVERSITY

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Abstract

Physiologic and Molecular Basis of Dry Land Adaptability in *Echinochloa* Species

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Echinochloa species is distributed around the world and regarded as one of the most problematic weeds because of its high competitiveness against crop and ecological adaptability. In Korea, two *Echinochloa* species, *E. oryzicola* (2*n*=4X) and *E. crus-galli* (2*n*=6X), are known to inhabit crop lands. Interestingly, each *Echinochloa* species inhabits a different habitat: *E. oryzicola* inhabits flooded paddy fields, while *E. crus-galli* mainly inhabits upland area, particularly *E. crus-galli* var. *praticola*. It is assumed that the different habitats of the two *Echinochloa* species may be related to the difference in their adaptability to osmotic stress. Therefore, this study was conducted to investigate the adaptability of *Echinochloa* species, such as *E. colona*, *E. crus-galli*, *E. oryzicola* and *E. oryzoides* collected from different habitats, to osmotic stress induced by polyethylene glycol (PEG) and NaCl, which were used to mimic osmotic stress conditions. Plant response to each osmotic stress was investigated at various growth stages of the *Echinochloa* species including germination (petri-dish assay), seedling emergence (growth pouch assay), and early juvenile plant growth (pot assay). In this study, seed germination test revealed that *E. colona* was the most tolerant to osmotic stress, maintaining high germination rate even at high PEG and NaCl concentration, while *E. oryzicola* (USA) was the most sensitive. Seedling emergence test revealed that *E. colona* and *E. crus-galli* var. *praticola* (only PEG) had higher root/shoot (R/S) ratio than *E. oryzicola* and *E. oryzoides*, in high PEG and NaCl concentration, suggesting that greater R/S ratio of *Echinochloa* is related to its adaptation to dry upland condition. Juvenile plant growth test revealed that the tolerant species maintained their growth ability and plant homeostasis in osmotic stress conditions. For instance, fresh weight, plant temperature and chlorophyll fluorescence of the most sensitive species, *E. oryzoides* changed much more than those of the most tolerant species, *E. colona*. At the molecular level, tolerant species and sensitive species showed difference in the expression profiles of genes related to drought tolerance mechanism such as enzymes included in ABA synthesis pathway and salt overly sensitive (*SOS*) pathway. In conclusion, our results demonstrate that the different adaptability of *Echinochloa* to osmotic stress enables *Echinochloa* species widely distribute at various crop lands with different water regimes.

Keywords: adaptive diversity, *Echinochloa*, drought, molecular mechanism, salt stress

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1. INTRODUCTION

Consisting of more than 50 species, *Echinochloa* is a very widespread genus of grass throughout the world (Flora of North America Editorial Committee, 2003). While some of its members could be cultivated as cereal or fodder crops, it has been generally known as a nuisance to us because they usually appear as a noxious weed conflicting with human interests.

It is known that *E. crus-galli*, hexaploid and the most widely distributed species, is evolved by natural hybridization between tetraploid *E. oryzicola* and unknown diploid *Echinochloa* species and duplication (Yabuno, 1983). *Echinochloa oryzicola* inhabits paddy field but not upland field, while *E. crus-galli* inhabits a broad range of habitats from paddy field to upland field, depending on variety and ecotype. For example, *E. crus-galli* var. *crus-galli* can be found in both paddy and upland fields, while *E. crus-galli* var. *praticola* almost always in upland field (Yamasue et al., 1989b). These findings indicate that they have differently adapted to the environment, particularly in accordance with water availability.

Some previous studies investigated how *E. oryzicola* could inhabit flooded paddy field, while *E. crus-galli* could not in terms of physiological and molecular aspects (Im, 2016; Nah et al., 2015). However, there is almost no research about the opposite aspect why *E. crus-galli*, particularly *E. crus-galli* var. *praticola*, can inhabit upland area, while *E. oryzicola* cannot, due to the difficulty of inducing and managing drought stress. Understanding the adaptive diversity in *Echinochloa* species may help understand their evolutionary mechanism and effectively manage *Echinochloa* species in crop lands.

Plant adaptation to stress environment is known to be influenced by its various physiological stages such as germination, seedling emergence, early juvenile plant growth, and reproductive growth stages. Therefore, this study was conducted to investigate adaptive diversity of *Echinochloa* species to osmotic stress and molecular mechanisms behind such adaptive diversity. We investigated and compared the characteristics of germination, seedling emergence, and early juvenile plant growth of *Echinochloa* species under osmotic stress conditions induced by polyethylene glycol (PEG) and NaCl. Expression profiles of drought-related genes associated with abscisic acid (ABA) synthesis, salt overly sensitive (SOS) pathway, and upstream components of signal transduction were also investigated to uncover molecular mechanism of adaptive diversity to osmotic stress in *Echinochloa* species.

2. LITERATURE REVIEW

2.1. *Echinochloa spp.*

Echinochloa is a very widespread genus of grass throughout the world, inhabiting from a tropical to warm-temperate region (Flora of North America Editorial Committee, 2003). The genus consists of about 50 species (Holm et al., 1977), and especially six of them are known to prevail worldwide including *E. oryzoides*, *E. oryzicola*, *E. crus-galli* and *E. colona* (Michael, 1983). While some of them have been cultivated as cereal or fodder crops, most *Echinochloa* spp. usually appear as noxious weeds conflicting with human interests (Hill et al., 1985; Moon et al., 2014; Moon et al., 2010).

2.2.1. *Echinochloa* spp. in Korea

More than eight species are distributed throughout Korea, including *E. crus-galli* var. *crus-galli, E. crus-galli* var. *echinata, E. crus-galli* var. *praticola, E. oryzicola, E. oryzoides, E. colona, E. glabrescens,* and *E. esculenta* (Lee et al., 2013). Among the *Echinochloa* spp., *E. oryzicola* and *E. crus-galli* are mainly found throughout the whole country and disturb crop production in Korea.

Morphologically, *E. oryzicola* and *E. crus-galli* are distinguished by plant form and seed morphology; The culm of *E. oryzicola* is erect and densely tufted even at maturity, while that of *E. crus-galli* is sprawling, decumbent or erect (Flora of North America Editorial Committee, 2003). Only *E. oryzicola* has lower glume which is larger than 3/5 of the length of the spikelet (Yabuno, 1983). The spikelet of *E. oryzicola* is larger than that of *E. crus-galli* in size, and it has short or no awn, compared to various awn length in *E. crus-galli*. Cytogenetically, *E. oryzicola* is tetraploid and *E. crus-galli* is hexaploid (Im, 2015; Yabuno, 1983).

Moreover, there are differences in habitats between the two species as well as morphological or cytogenetic differences. *Echinochloa oryzicola* inhabits paddy field but not upland field, while *E. crus-galli* inhabits a broad range of habitats from paddy to upland field, depending on variety and ecotype (Nah et al., 2015). For example, *E. crus-galli* var. *crus-galli* can be found in both paddy and upland fields, while *E. crus-galli* var. *praticola* almost always in upland field (Yamasue et al., 1989a & 1989b).

2.2. Drought stress

Physiologically, drought stress can be usually applied to plants through water deficit, which can be defined as moisture content of a tissue or cell under the highest moisture content in the most hydrated state (Taiz and Zeiger, 2010). However, abundant solution with relatively lower water potential than the interior of plant, such as sodium chloride solution, can also induce water deficit condition (Hamada, 1996; Mohammadkhani and Heidari, 2008; Zhu, 2007) in that plants cannot absorb water inward in spite of different mechanism.

2.2.1. Plant response under drought stress

Reducing water uptake in water deficit conditions reduces tissue moisture content. Therefore, the turgor is lost and cell elongation is suppressed, resulting in reduced leaf area. Stomata are closed to reduce evaporation in the existing leaf area in response to increased abscisic acid. Water deficit also increases wax deposition on leaf surfaces and limits photosynthesis in chloroplasts (Farooq et al., 2009). Meanwhile, exposing plants to environmental stresses often produces reactive oxygen species. These ROS can cause oxidative damage and impair normal cell function (Foyer and Fletcher, 2001; Munne-Bosch and Penuelas, 2003). Moreover, slight water deficit affects the development of the root system, causing roots to expand into deeper and damper soil (Taiz and Zeiger, 2010).

2.2.2. How to induce drought stress

Drought stress is usually induced by adding osmotica, such as polyethylene glycol (PEG) and mannitol. PEG is a neural polymer (Lawlor, 1970). PEG of large molecular weight (4,000~8,000) hardly penetrates the plant cell membranes but effectively modifies water potential of root-around media (Blum, 2008; Gangopadhyay et al., 1997). By the way, mannitol is a natural product that presents in plant cells in the certain amount, and its effect as a drought inducer is similar to that of PEG. However, mannitol has a critical defect as a drought inducer because mannitol is small enough to cross the cell membrane (Fritz and Ehwald, 2011; Lipavská and Vreugdenhil, 1996).

NaCl also lower the water potential of root-around media (Mohammadkhani and Heidari, 2008), because Na^+ and Cl^- ions directly change the concentration of the media, influencing the water potential. Moreover, NaCl can also cause ionic stress, because high concentrations of Na^+ in plants can be toxic (Munns and Tester, 2008). Therefore, it is difficult to see the effect of NaCl as only that of drought stress, but NaCl can sometimes be a nice substitute (Claeys et al., 2014).

2.3. Drought tolerance test methods

2.3.1. Germination stage

Water is one of the essential elements for seed germination with light, temperature, oxygen. Therefore, the excess or lack of water can affect seed germination. Moreover, higher germination rate is directly and indirectly related to higher survival rate, because high germination rate can give high chance of survival.

Echinochloa oryzicola showed higher germination rate than *E. crus-galli* in deep water condition (Kim, 1993), suggesting that *E. oryzicola* better adapts to flooded condition than *E. crus-galli*. Similar results can also be found in Yamasue's research (Yamasue et al, 1989a). *E. oryzicola* and *E. crus-galli* var. *formosensis* showed 90 and 80% germination rate respectively, while *E. crus-galli* var. *praticola* did not germinate at the flooded soil.

As NaCl concentration increased, the germination rate of *E. colona* decreased linearly. Germination rate of *E. colona* was greater than 60% up to the concentration of 50 mM NaCl, while inhibited completely at 200 mM NaCl. When it comes to osmotic stress induced by PEG 8000, the pattern of germination decrease was different. However, as the PEG concentration increased, the rate of germination dropped to 0% at 21.0 MPa, following sigmoidal curve (Chauhan and Johnson, 2009). Similar germination inhibition results could be observed in *E. glabrescents* (Opeña et al., 2014).

2.3.2. Seedling emergence stage

Growth pouch method (Zhang et al., 2015) helps to investigate the changes in root and shoot length under abiotic stress condition. When it comes to root/shoot length (R/S) ratio, it is helpful for plants to keep R/S ratio low during sufficient water condition. However, during limited water condition, plants immediately change their strategy to ensure the sufficient amount of water, minimizing shoot growth and maintaining or promoting root growth. This allows plants to avoid or minimize leaf water loss as well as maximize water uptake in the soil (Taiz and Zeiger, 2010).

In Park's research (Park et al., 2016), root and shoot growth of all the tested *Echinochloa* species were inhibited, with PEG concentration increasing . Moreover, PEG treatment affected R/S ratio of *E. oryzicola* and *E. crus-galli*. In particular, *E. crus-galli* var. *praticola* increased R/S ratio as the osmotic stress increased, while *E. oryzicola* decreased R/S ratio or showed smallest R/S ratio.

2.3.3. Early juvenile plant growth stage

Inhibition of leaf growth is a kind of primary plant's response to moderate water stress (Lu and Neumann, 1998; Taiz and Zeiger, 2010). The different water potential gradient between expanding cells and the surrounding water source, can directly reduce cell expansion (Kramer and Boyer, 1995; Nonami, 1998), which eventually limits transpirational water loss with leaf abscission.

In Yamasue's research (Yamasue et al., 1989b), the photosynthesis/transpiration (PS/TR) ratio to drought stress was compared among 4 *Echinochloa* spp. The PS/TR ratio of *E. crus-galli* var. *praticola* was three times higher than that of *E. oryzicola*, resulting from much higher transpiration rate of *E. oryzicola* and much higher stomatal resistance of *E. crus-galli* var. *praticola*.

Thermal image analysis is the most common technique for visualizing temperature differences. Infrared thermal image is also commonly used to measure the osmotic stress caused by salinity or drought in cereal crops (Yang et al., 2013). Chen (Chen et al, 2005) used thermal image analysis to examine drought responses in corn and soybean and Kim (Kim et al, 2014) to screen salt-tolerant soybean. Moreover, leaf temperature is useful for estimating stomatal movement (Jossier et al., 2010), stomatal conductance (Jones, 1999; Kim et al., 2014), and transpiration rate (Katul et al., 2000).

Fluorescence images are used to analyze the maintenance of photosynthesis function and to assess the early crop response to abiotic stress, such as drought (Baker, 2008), even when crops do not exhibit visual symptoms (Lee, 2015). Fv/Fm, which is the most commonly used parameter, is aimed at measuring the photosynthetic efficiency of photosystem II (Misra et al., 2012) in a dark-adapted state and is significantly correlated with photosynthesis under salt stress (Kim et al., 2014).

In addition to shoot- or leaf-related traits, deep and extensive root growth is also a kind of survival strategy for osmotic stress. This is because increasing water extraction through root is as important as reducing water loss through leaves (Fukai and Cooper, 1995; Serraj et al., 2004; Yamauchi et al., 1994).

2.4. Molecular mechanism of drought tolerance

Abscisic acid (ABA) is a kind of phytohormone that regulates plant growth and developmental processes under environmental stress conditions. High levels of ABA mean that both ABA biosynthesis and catabolism are active in plants. There are many enzymes involved in ABA biosynthetic pathway, such as ZEP, NCED, ADH, and AAO (Barrero et al., 2006; Finkelstein, 2013; Hadiarto and Tran, 2011). Likewise, one of the major ways for ABA catabolism in *Arabidopsis* is a hydroxylation of ABA at the 8′ position by cytochrome P450 monooxygenases, which *CYP707A* families encode (Kushiro et al., 2004; Saito et al., 2004; Shinozaki and Yamaguchi-Shinozaki, 2007; Verslues, 2016). Through quantifying these enzymes or enzymatic activity, the amount of ABA biosynthesis can be indirectly expected.

In addition to hormonal changes to regulate physiologic responses of plants, it is also necessary to re-establish internal states of plants to maintain ion homeostasis. For example, salt overly sensitive (SOS) pathway is essential for saline stress tolerance. Under saline stress, the sensor protein SOS3 detects a rise in the cytoplasmic free calcium concentration and activates the protein kinase SOS2 which forms a complex with itself. Then, *SOS1*, which encodes Na^+-H^+ antiporter, is phosphorylated and Na^+ concentration in cytosol remains constant (Taiz and Zeiger, 2010; Zhu, 2002) In addition to SOS related genes, proton pumps in the tonoplast, such as vacuolar-H^+-ATPase helps to generate the electrochemical gradient for secondary transport of ions into the vacuole. Through these genes, plants have the ability to control ion concentration in the cytoplasm, maintaining homeostasis.

There is also signal transduction pathway, such as protein kinases (Todaka et al., 2015) and transcription factors (Agarwal and Jha, 2010; Hadiarto and Tran, 2011) that regulate stress-inducible gene expression in upstream level. For example, *NPX1*, *MYB15*, and *CPK*s are protein kinases, which are known to function to control opening and closing of stomata (Boudsocq and Sheen, 2013; Cominelli et al., 2010; De Leonardis et al., 2012).

Moreover, mechanisms for ensuring sufficient water absorption are activated, because both drought and salt-induced osmotic stress cause water shortage condition. Through accumulation of compatible solutes, such as proline and sugar alcohols, the osmotic potential of cell is lowered, and plants can attract water into the cell.

3. MATERIALS AND METHODS

3.1. Plant materials

Six *Echinochloa* species, *Echinochloa oryzicola*, *E. crus-galli* var. *crus-galli*, *E. crus-galli* var. *praticola*, *E. oryzoides* and *E. colona*, were collected from different sites in Korea, USA and Costa Rica between 1993 and 2014 (Table 1).

Table 1. *Echinochloa* species used in this study

Scientific name	Collection site	Collection year
Echinochloa oryzicola	Pocheon, Korea	2011
Echinochloa crus-galli var. *crus-galli*	Cheolwon, Korea	2011
Echinochloa crus-galli var. *praticola*	Suwon, Korea	2001
Echinochloa oryzicola	California, USA	2014
Echinochloa oryzoides	California, USA	2014
Echinochloa colona	Costa Rica	1993

3.2. Germination test (petri-dish assay)

Each *Echinochloa* seed was soaked in each petri-dish with a range of drought stress. PEG 6000 and NaCl were used to induce drought stress and 6 ranges of concentrations were used in each treatment; 0, 40, 60, 80, 120, 160, 240 g PEG L^{-1} and 0, 25, 50, 100, 150, 200 mM NaCl. All petri-dishes were incubated in a growth chamber (Hanbaek Scientific Lt., Korea) at the temperature 24/20^{o}C and 16/8 hours of photo-period (day/night) during 6 days after sowing. All the petri-dishes were watered daily as much as the amount of evaporation and seed absorption. The number of germinated seeds was recorded every 12 hours and the germination rate was calculated in each species based on PEG and NaCl concentration gradient. The experiment consisted of 4 replications.

3.3. Seedling emergence test (growth pouch assay)

Echinochloa seeds were soaked in each petri-dish after 3 days of priming treatment. As soon as the seeds germinated, the germinated seeds were placed at the growth pouch. Each growth pouch contained 9 mL of PEG or NaCl solution and was watered as much as the amount of evaporation and seed absorption. Six ranges of concentrations were used in each treatment; 0, 40, 80, 120, 160, 240 g PEG L^{-1} and 0, 25, 50, 100, 150, 200 mM NaCl. All growth pouches were incubated in a growth chamber (Hanbaek Scientific Lt., Korea) at the temperature 28/24ºC and 16/8 hours of photo-period (day/night) during 6 days after transplanting. The length of shoot and root was recorded every day and the root/shoot length (R/S) ratio was calculated in each species based on PEG and NaCl gradient. The experiment consisted of 4 replications and 1 replication contained 5 plants.

3.4. Early juvenile plant growth test (pot assay)

Each *Echinochloa* seed was soaked in each petri-dish in after 3 days of priming treatment and maintained in a growth chamber (Hanbaek Scientific Lt., Korea) at the temperature 24/20ºC and 16/8 hours of photo-period (day/night). After germination, plants were grown in a multiple plastic pot up to 4~5 leaf stage in glasshouse maintained at the experimental farm station of SNU with regular irrigation. At the 4~5 leaf stage, seedling will be transplanted to a circular plastic pot containing soil with a range of PEG and NaCl concentrations; 0, 40, 60, 80, 120, 160 g PEG L^{-1} and 0, 25, 50, 100, 150, 200 mM NaCl. After transplanting, visual assessment was carried out and then, to accurately measure physiologic response of plants, RGB image, thermal image, and chlorophyll fluorescence image were taken at 2 days after PEG and NaCl treatment. Finally, fresh shoot weight was measured at 3 days after PEG and NaCl treatment.

3.5. Gene expression analysis with quantitative real time PCR

The two most tolerant *E. colona*, and *E. crus-galli* var. *praticola* and the two most sensitive *E. oryzicola* (Korea) and *E. oryzoides* were grown in the same circumstance as the pot assay. Only one concentration of stress was treated in both PEG and NaCl treatment, which was lethal concentration of sensitive species, 100 g PEG L^{-1} and 80 mM NaCl. The experiment was conducted with four biological replicates including three-plant pooling.

For qRT-PCR analysis, the only leaf parts of each *Echinochloa* were sampled at fourth leaf stage. Total RNA was extracted from 100 mg of tissue using Hybrid-R™ and Riboclear™ plus kits (GeneAll, Korea). Quantity and quality of total RNA were confirmed using both Nanodrop 2000 spectrophotometer (Nanodrop, USA) and gel electrophoresis (Takara, Japan). cDNA was synthesized from 500 ng of total RNA using TOPscript Reverse Transcriptase (Enzynomics, Korea). qRT-PCR was carried out using TOPreal™ qPCR 2X PreMIX for SYBR Green (Enzynomics, Korea) with a Rotergene Q real-PCR machine (Qiagen, US). The qRT-PCR was initially set for 15 min at 95°C, followed by 45 cycles of 95°C for 10 sec for denaturation, 60°C for 15 sec for annealing, and 72°C for 20 sec for a final extension. *Actin* gene was used as a housekeeping gene for the normalization of each *Echinochloa* spp. Relative gene expression level was calculated using delta-delta C_t method (Livak and Schmittgen, 2001) with two technical and three biological replicates.

3.6. Statistical analyses

Non-linear regression analysis was conducted by fitting the log-logistic model (Streibig, 1980) to the germination rate and the fresh weight at harvest.

$$Y = \frac{Y_o}{1 + (\frac{Dose}{GR_{50}})^B}$$

where, Y_o is 100, because % control value was used, GR_{50} refers to the PEG or NaCl concentration at which the germination or growth was reduced by 50% of the untreated control, and *B* refers the slope of the standard dose-response curve.

Analysis of variance (ANOVA) was conducted for R/S ratio, plant temperature, chlorophyll fluorescence, and RNA expression. It was followed by the Tukey-HSD for post-hoc comparisons ($P<0.05$). All statistical analyses were conducted using SAS software version 9.4 of the SAS System for Windows® (SAS Institute Inc., USA).

4. RESULTS

4.1. Adaptive diversity in germination

During germination stage, osmotic stress was induced by 6 ranges of each PEG and NaCl treatment. Figure 1 showed the germination rate of each *Echinochloa* spp. at 4.5 days after PEG treatment. With low PEG treatment, no significant germination reduction was observed, and even slight stimulation of germination was observed in some species, such as *E. oryzicola* (Korea) and *E. colona*, compared to control (0 g PEG L^{-1}). In the case of high PEG treatment, *E. colona* and *E. crus-galli* var. *crus-galli* maintained their germination patterns relatively, compared to the other species, leading to high GR_{50} value.

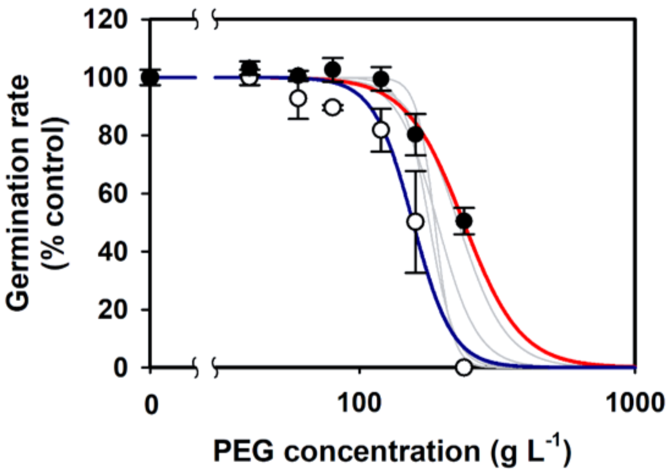


Figure 1. Germination rate and dose response curves of *Echinochloa* species showing maximum and minimum tolerance. *E. colona* (●, red line) and *E. oryzicola* from USA (○, blue line) with PEG-induced stress. Gray lines indicate the other *Echinochloa* species.

The GR_{50} value in germination investigation means the concentration at which the germination rate is reduced by 50%. The GR_{50} value of *E. colona* and *E. curs-galli* var. *crus-galli* is 239.0 and 228.1 g PEG L^{-1}, respectively, which is significantly different from that of *E. oryzicola* (USA), 156.7 g PEG L^{-1} (Table 2).

Table 2. The GR_{50} values in germination rate of 6 *Echinochloa* spp. in response to PEG-induced stress. Numbers in parentheses are standard error

***Echinochloa* spp.**	*E. colona*	*E. crus-galli* var. *praticola*	*E. crus-galli* var. *crus-galli*	*E. oryzicola* (Korea)	*E. oryzicola* (USA)	*E. oryzoides*
GR_{50}	239.0 (6.4)	179.2 (6.9)	228.1 (4.8)	192.0 (4.1)	156.7 (4.7)	187.7 (1.7)

Moreover, osmotic stress was also induced by 6 ranges of NaCl treatment. Figure 2 showed the germination rate of each *Echinochloa* spp. at 4.5 days after NaCl treatment. The response of NaCl treatment was different from that of PEG treatment. Germination decline was clearly observed throughout the NaCl concentration, as the concentration of NaCl treatment increased. However, as with the response of PEG treatment, *E. colona* retained their germination patterns and *E. oryzicola* (USA) failed to maintain their germination patterns compared to the other species, at high NaCl concentration around 100 mM.

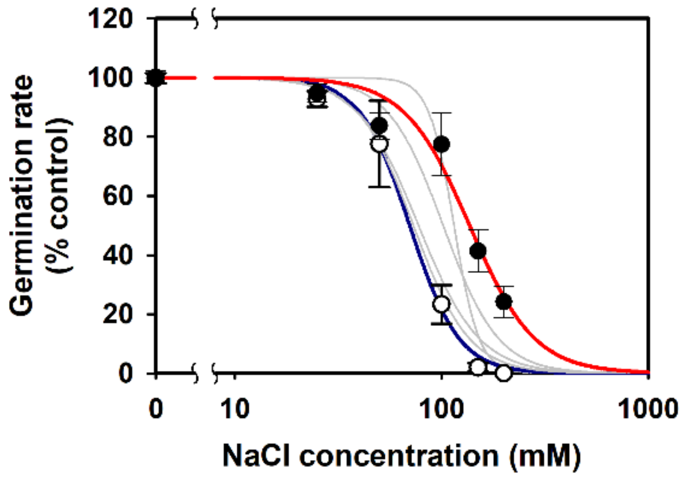


Figure 2. Germination rate and dose response curves of *Echinochloa* species showing maximum and minimum tolerance. *E. colona* (●, red line) and *E. oryzicola* from USA (○, blue line) with NaCl-induced stress. Gray lines indicate the other *Echinochloa* species.

The GR_{50} value of *E. colona* and *E. oryzoides* is 136.6 and 114.9 mM NaCl, respectively. In particular, the GR_{50} value of *E. colona* is almost twice GR_{50} value of *E. crus-galli* var. *crus-galli*, *E. crus-galli* var. *praticola* and *E. oryzicola* (USA), 77.4, 73.1 and 70.1 mM NaCl, respectively (Table 3).

Table 3. The GR_{50} values in germination rate of 6 *Echinochloa* spp. in response to NaCl stress. Numbers in parentheses are standard error

***Echinochloa* spp.**	*E. colona*	*E. crus-galli* var. *praticola*	*E. crus-galli* var. *crus-galli*	*E. oryzicola* (Korea)	*E. oryzicola* (USA)	*E. oryzoides*
GR_{50}	136.6 (6.2)	73.1 (3.2)	77.4 (5.1)	101.8 (6.7)	70.1 (3.2)	114.9 (3.1)

4.2. Adaptive diversity in seedling emergence

Root to shoot (R/S) ratio of *E. colona* increased by both PEG- and NaCl-induced osmotic stress, while that of *E. crus-galli* var. *praticola* increased only by PEG-induced osmotic stress (Figure 3 and 4). Meanwhile, R/S ratio of *E. oryzoides* decreased by both PEG- and NaCl-induced osmotic stress. Other *Echinochloa* species showed slightly lower or similar R/S ratios with increasing concentration of both stress treatments.

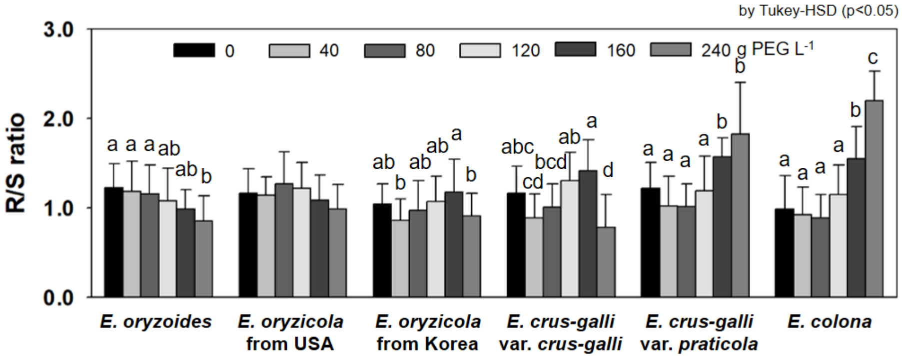


Figure 3. Root to shoot (R/S) ratios of *Echinochloa* species grown in the PEG-containing growth pouch. The same letter indicates no significant difference and different letters indicate significant differences at *P*<0.05 determined by Tukey-HSD.

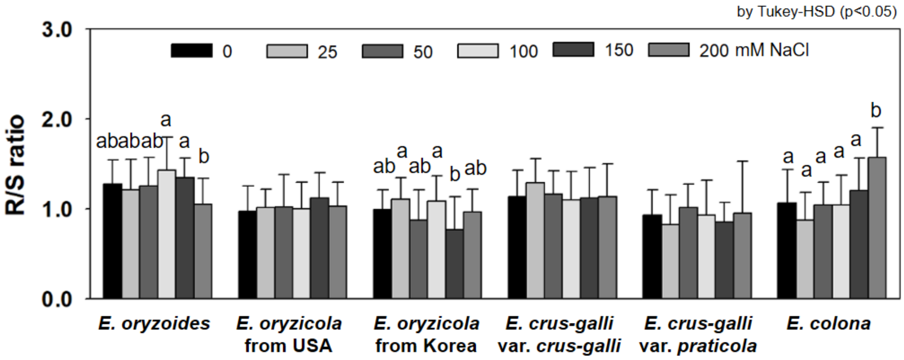


Figure 4. Root to shoot (R/S) ratios of *Echinochloa* species grown in the NaCl-containing growth pouch. The same letter indicates no significant difference and different letters indicate significant differences at $P<0.05$ determined by Tukey-HSD.

4.3. Adaptive diversity in early juvenile plant growth

Figure 5 showed the response of each *Echinochloa* spp. on 2 days after PEG treatment. The growth of *E. colona* and *E. crus-galli* var. *praticola* was maintained similarly to control (0 g PEG L^{-1}) under 120 g PEG L^{-1} treatment. However, *E. oryzicola* (USA) and *E. oryzoides* failed to keep growing as well as control, causing leaf curling and limp-looking leaves, even under 60 g PEG L^{-1} treatment. *E. crus-galli* var. *crus-galli* showed intermediate responses against PEG treatment, with a lethal concentration around 120 g PEG L^{-1}.

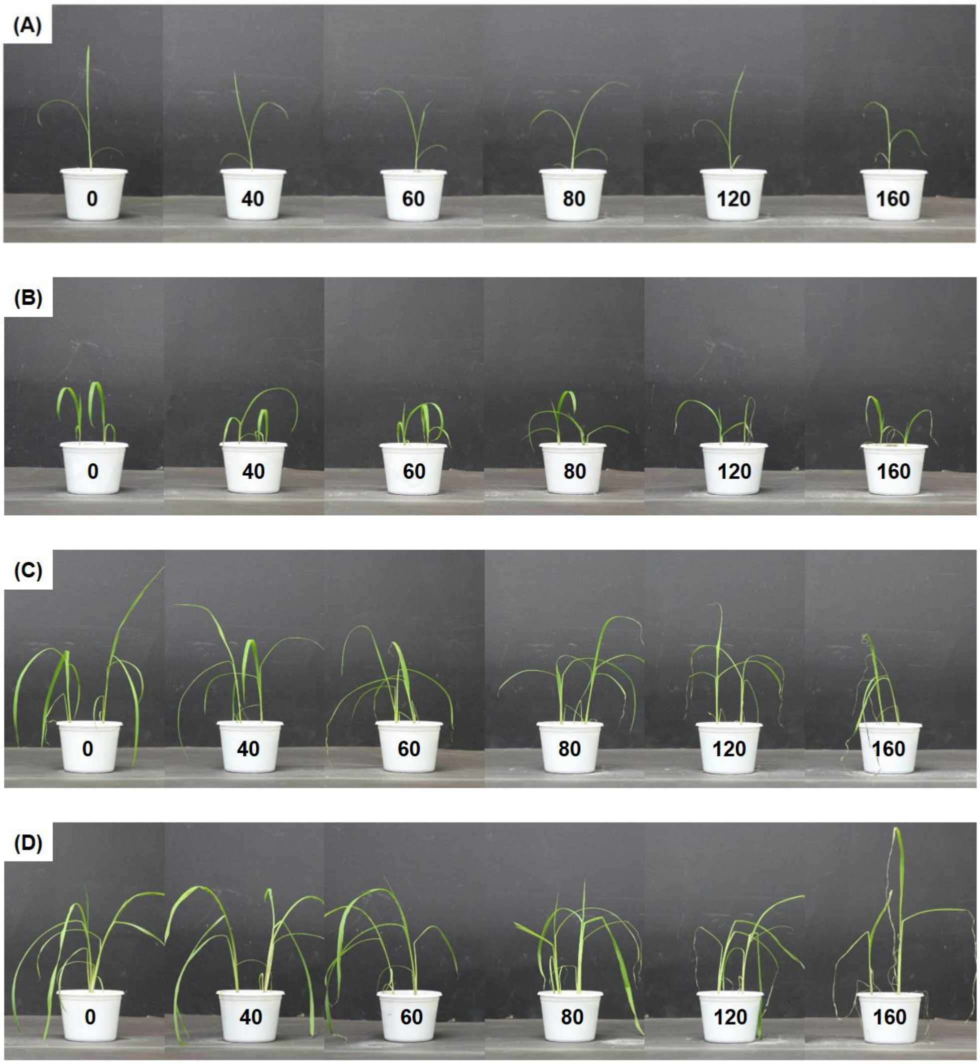


Figure 5. *Echinochloa* spp. at two days after PEG treatment. The most PEG tolerant two *Echinochloa* spp., *E. colona* (A) and *E. crus-galli* var. *praticola* (B), the most PEG sensitive two species, *E. oryzicola* from USA (C) and *E. oryzoides* (D).

Figure 6 and Table 4 showed the fresh weight of 6 *Echinochloa* species on 3 days after PEG treatment. It also supported upper results, as *E. colona* and *E. crus-galli* var. *praticola* kept their fresh weight at higher PEG concentrations with high GR_{50} value.

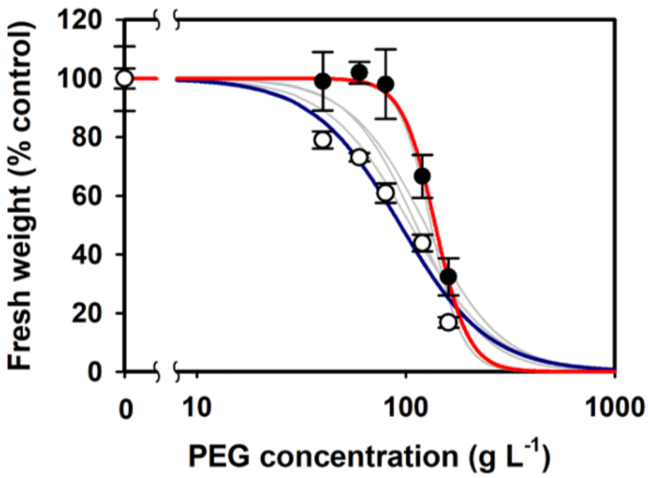


Figure 6. Fresh weight and dose response curves of *Echinochloa* species showing maximum and minimum tolerance. *E. colona* (●, red line) and *E. oryzoides* (○, blue line) with PEG-induced stress. Gray lines indicate the other *Echinochloa* species.

Table 4. The GR_{50} values in fresh weight of 6 *Echinochloa* spp. in response to PEG-induced stress. Numbers in parentheses are standard error

***Echinochloa* spp.**	*E. colona*	*E. crus-galli* var. *praticola*	*E. crus-galli* var. *crus-galli*	*E. oryzicola* (Korea)	*E. oryzicola* (USA)	*E. oryzoides*
GR_{50}	138.4 (4.4)	132.1 (6.2)	124.3 (5.9)	105.4 (5.6)	112.2 (4.2)	94.3 (3.8)

Figure 7 showed the thermal images of the most tolerant species, *E. colona* and the most sensitive species, *E. oryzoides*. Both species showed similar plant temperature up to 40 g PEG L^{-1} compared to control (0 g PEG L^{-1}). However, they showed clear temperature difference from 80 g PEG L^{-1}. The temperature change of *E. colona* was negligible, whereas that of *E. oryzoides* changed noticeably with an especially high temperature increase around leaf tip. Figure 8 showed the plant temperature of all the *Echinochloa* species. The temperature of *E. oryzoides*, *E. oryzicola*, and *E. crus-galli* var. *crus-galli* increased significantly with increasing PEG treatment. However, that of *E. colona* and *E. crus-galli* var. *praticola* was kept constant with no statistical difference.

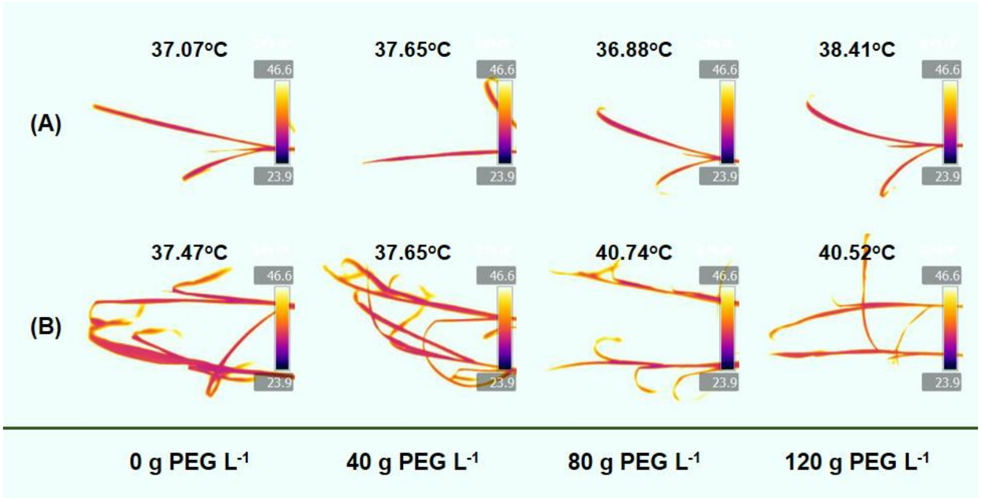


Figure 7. Thermal image of the most tolerant, *E. colona* (A) and the most sensitive species, *E. oryzoides* (B) to PEG-induced stress.

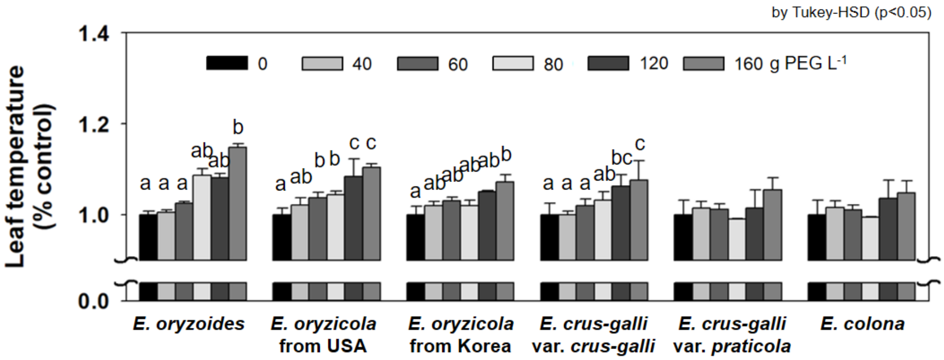


Figure 8. Plant temperature of *Echinochloa* species treated with PEG. The same letter indicates no significant difference and different letters indicate significant differences at P<0.05 determined by Tukey-HSD.

Figure 9 showed the Fv/Fm value calculated from chlorophyll fluorescence image. The Fv/Fm value of *E. oryzoides* and *E. oryzicola* decreased with increasing PEG concentration, while that of the other species tends to decrease with increasing PEG concentration with no statistically significant difference.

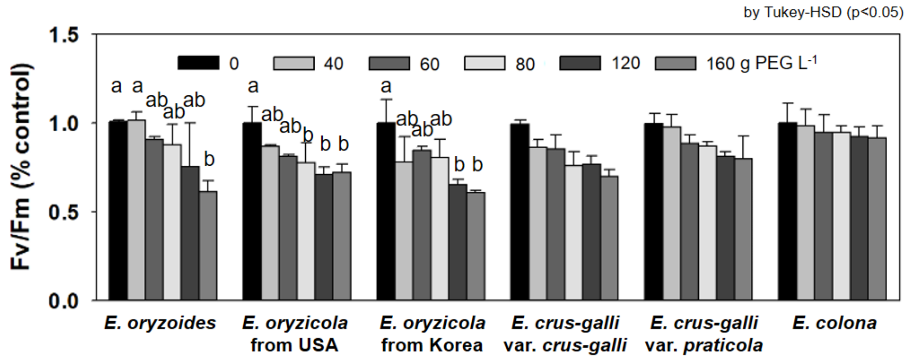


Figure 9. Chlorophyll fluorescence of PEG-treated *Echinochloa* spp. The same letter indicates no significant difference and different letters indicate significant differences at $P<0.05$ determined by Tukey-HSD.

Figure 10 showed the response of each *Echinochloa* spp. on 2 days after NaCl treatment. In spite of slight growth reduction, no clear NaCl-induced stress response, such as chlorosis and wilting, was observed in *E. colona* even by 200 mM NaCl treatment. The growth of *E. crus-galli* var. *praticola* was maintained similarly to control under 100 mM NaCl treatment. However, the other species failed to keep growing as well as control (0 mM NaCl) under 100 mM NaCl treatment, showing more than 50% leaf blight at 150mM.

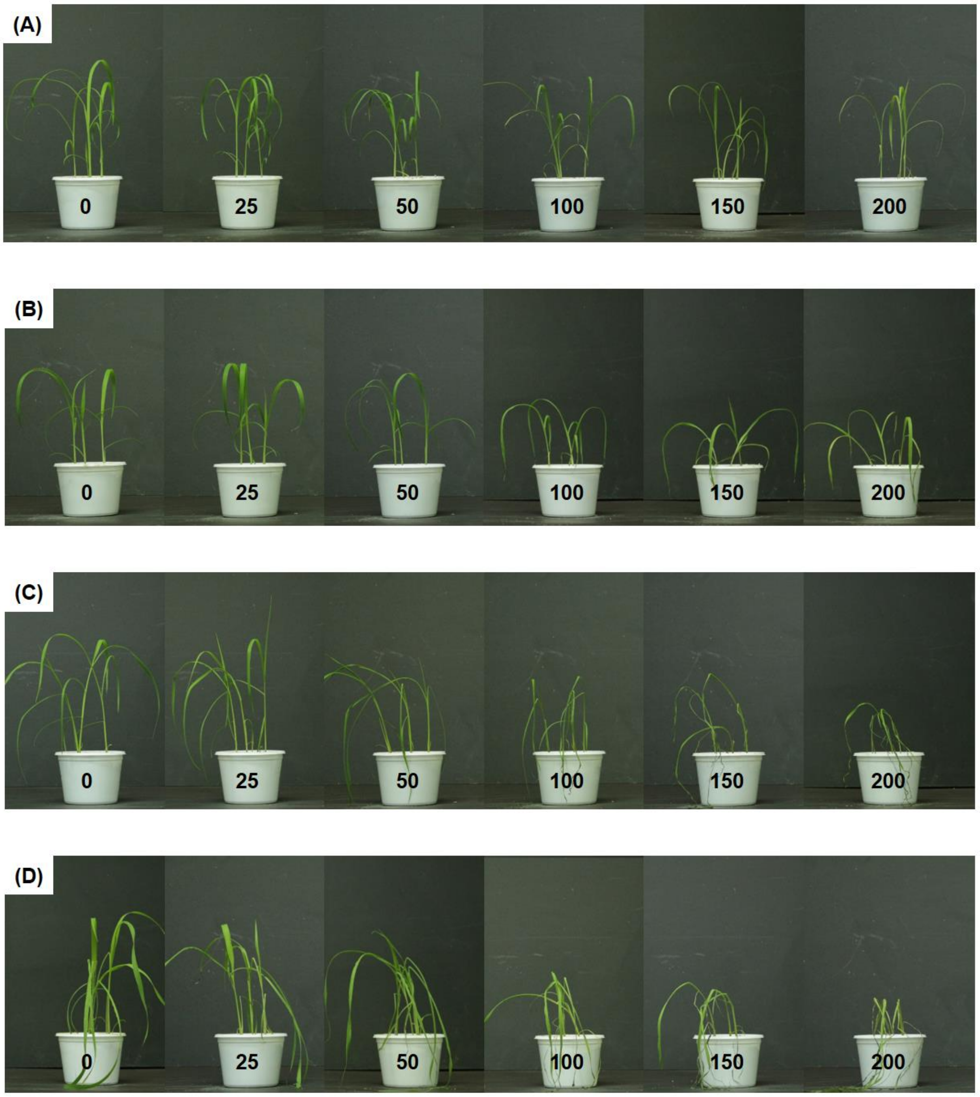


Figure 10. *Echinochloa* spp. at two days after NaCl treatment. The most NaCl tolerant two *Echinochloa* spp., *E. colona* (A) and *E. crus-galli* var. *praticola* (B), the most NaCl sensitive two species, *E. oryzicola* from USA (C) and *E. oryzoides* (D).

Figure 11 showed the fresh weight of 6 *Echinochloa* species on 3 days after NaCl treatment. Except *E. colona* and *E. crus-galli* var. *praticola*, other species began to lose their fresh weight compared to control (0 mM NaCl) even at 25 mM NaCl treatment. *E. colona* and *E. crus-galli* var. *praticola* showed high GR_{50} value while *E. oryzicola* and *E. oryzoides* showed low GR_{50} value, supporting previous RGB image results (Table 5).

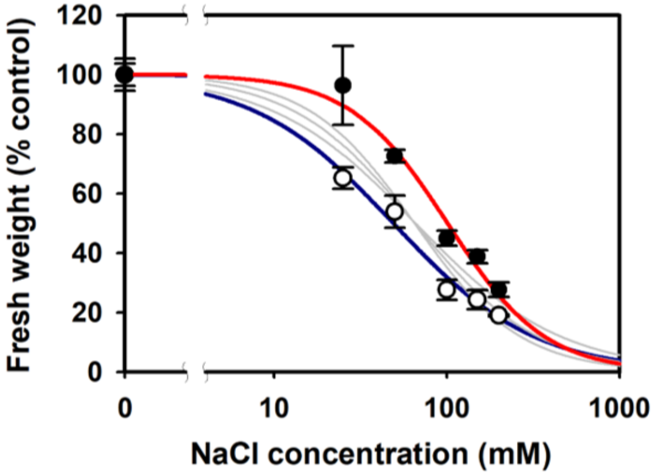


Figure 11. Fresh weight and dose response curves of *Echinochloa* species showing maximum and minimum tolerance. *E. colona* (●, red line) and *E. oryzoides* (○, blue line) with NaCl-induced stress. Gray lines indicate the other *Echinochloa* species

Table 5. The GR_{50} values in fresh weight of 6 *Echinochloa* spp. in response to NaCl stress. Numbers in parentheses are standard error

Echinochloa spp.	*E. colona*	*E. crus-galli* var. *praticola*	*E. crus-galli* var. *crus-galli*	*E. oryzicola* (Korea)	*E. oryzicola* (USA)	*E. oryzoides*
GR_{50}	101.2 (7.1)	100.7 (7.6)	66.1 (5.1)	65.1 (3.5)	65.5 (4.8)	49.2 (3.2)

Figure 12 showed the thermal image of the most tolerant species and the most sensitive species to NaCl-induced stress, *E. colona* and *E. oryzoides* respectively. Both the temperature difference among both species began to diverge in 50 mM

NaCl.

Figure 13 showed the plant temperature of all the *Echinochloa* species. The temperature of all *Echinochloa* spp. increased as NaCl concentration increased. However, the pattern and degree of increase were different. At 100 mM NaCl which is close to lethal concentration of sensitive species, the temperature of sensitive species increased much more than that of tolerant species; the temperature of *E. oryzoides and E. oryzicola* increased 7.6~10.0% compared to control, whereas that of *E. colona* and *E. crus-galli* var. *crus-galli*, only increased 2.8~5.6% compared to control. At 200 mM NaCl, the temperature of *E. oryzoides*, the most sensitive species increased up to 15.0% while that of *E. colona*, the most tolerant species increased only 9%.

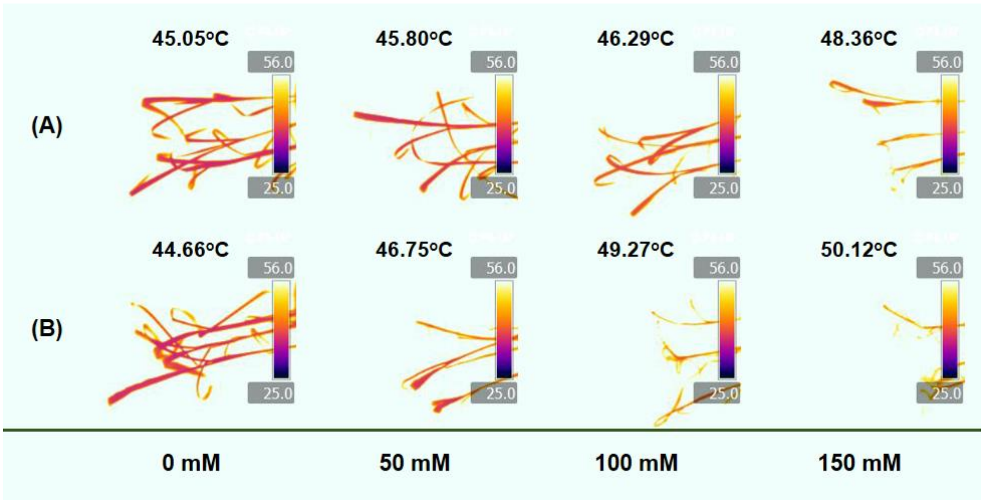


Figure 12. Thermal image of the most tolerant, *E. colona* (A) and the most sensitive species, *E. oryzoides* (B) to NaCl-induced stress.

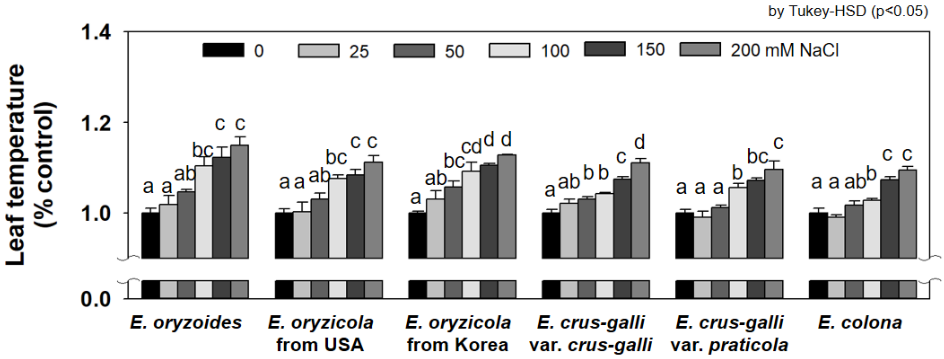


Figure 13. Plant temperature of *Echinochloa* species treated with NaCl. The same letter indicates no significant difference and different letters indicate significant differences at $P<0.05$ determined by Tukey-HSD.

Figure 14 showed the Fv/Fm value calculated from chlorophyll fluorescence image. The Fv/Fm value of all the *Echinochloa* species decreased significantly as the NaCl concentration increased. However, at 50 mM NaCl which is close to lethal concentration of sensitive species, the chlorophyll fluorescence of sensitive species decreased much more than that of tolerant species; the Fv/Fm value of *E. oryzoides*, the most sensitive species, decreased up to 50% compared to control (0 mM NaCl), whereas that of *E. colona*, the most tolerant species only decreased up to 70% compared to control. At 200 mM NaCl, the difference of Fv/Fm between *E. oryzoides* and *E. colona* reached 37%. However, *E. crus-galli* var. *praticola* did not show significantly different patterns compared to *E. oryzicola*.

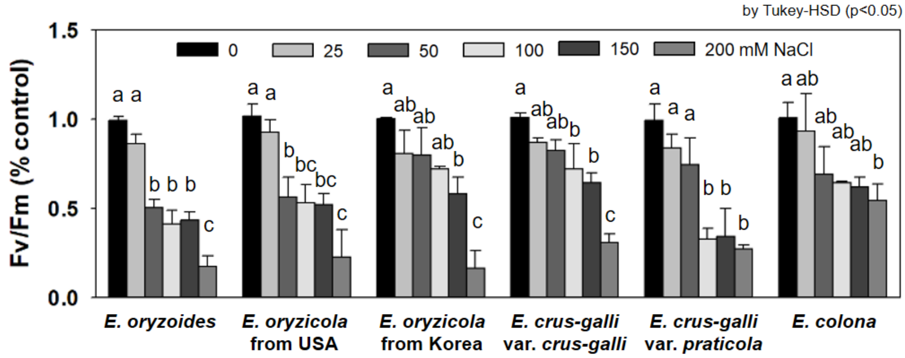


Figure 14. Chlorophyll fluorescence of NaCl-treated *Echinochloa* spp. The same letter indicates no significant difference and different letters indicate significant differences at $P<0.05$ determined by Tukey-HSD.

4.4. Molecular mechanism

Plant adaptation to environmental stress is related to a series of their endogenous stress tolerance mechanisms including signal perception, protein kinase cascade, transcriptional control, and gene expression. Table 6 contains the gene information related to osmotic stress and ionic toxicity tolerance (Munns and Tester, 2008), and tested in this experiment.

Table 6. Tested genes with regard to osmotic stress and ion toxicity

Functional group	Gene	description
ABA biosynthesis & catabolism	*ZEP*	Zeaxanthin epoxidase
	ADH	Alcohol dehydrogenase
	AAO3	Abscisic aldehyde oxidase 3
	CYP707A4	Cytochrome P450, family 707, subfamily A, polypeptide 4
Ion partition	*SOS1*	Sodium proton exchanger
	VHA-A1	Vacuolar proton ATPase A1
	KEA1	K+ efflux antiporter 1
Protein kinase	*SIP1,3*	SOS3-interacting protein
	CIPK12	CBL-interacting protein kinase 12
	CPK7	Calcium-dependent protein kinase
	MPK4	MAP kinase 4
	MYB15	MYB domain protein 15
	NPX1	Nuclear protein X1
Transcription factor	*DREB2A*	DRE-binding protein 2A
	bZIP23,44	Basic-leucine zipper (bZIP) transcription factor family protein
	ABI1,3	Protein phosphatase 2C family protein
	ABF4	ABRE binding factor 4
Other factors	*HST70*	Heat shock protein 70

Functional group	Gene description	PEG				NaCl			
		Oryzoides	Oryzicola	Praticola	Colona	Oryzoides	Oryzicola	Praticola	Colona
ABA biosynthesis & catabolism	*ZEP*	3.26	2.64	1.40	0.97	3.43	2.86	1.43	1.37
	ADH	1.21	1.11	1.21	0.93	1.25	1.28	1.71	1.02
	AAO3	-1.19	-0.90	-0.92	-0.87	1.06	1.21	1.27	0.91
	CYP707A4	4.00	3.55	2.06	1.45	4.35	3.81	2.24	2.10
Ion partition	*SOS1*	0.41	0.57	0.49	0.66	1.98	1.74	3.19	3.91
	VHA-A1	0.63	0.92	0.65	0.82	1.74	1.89	3.19	3.65
	KEA1	-0.14	0.01	-0.12	-0.03	0.62	0.92	0.88	0.77
Protein kinase	*SIP1*	0.18	0.25	0.04	-0.15	1.15	1.92	1.62	2.57
	CIPK12	1.21	1.27	1.31	1.25	0.98	1.19	0.96	1.03
	CPK7	1.29	1.32	1.14	1.32	0.99	1.28	0.99	1.39
	MPK4	0.58	0.43	0.63	1.07	0.58	0.52	1.00	1.41
	MYB15	2.19	1.91	1.97	1.84	2.05	1.69	1.90	1.91
	NPX1	0.61	0.56	0.36	0.38	1.04	1.09	0.93	0.94
Transcription factor	*DREB2A*	1.48	1.41	2.11	2.78	1.52	1.38	2.10	2.94
	bZIP23	-0.67	-0.43	-0.54	-0.33	-0.95	-0.62	-1.07	-0.32
	bZIP44	1.59	1.66	1.91	2.64	1.67	1.63	3.27	3.57
	ABI1	4.00	3.49	3.37	4.01	4.60	4.17	3.98	4.86
	ABI3	-1.03	-0.81	-1.34	-1.32	-0.90	-1.01	-1.36	-1.30
	ABF4	3.42	3.35	3.45	3.02	3.84	3.31	4.18	2.52
Other factors	*HST70*	1.72	0.96	3.32	3.05	1.57	1.27	3.08	3.62

Color scale: Min – Max

Figure 15. The expression pattern of genes in four *Echinochloa* species. Each value means the $\log_2$ fold changes in the expression level of each gene between 4 hours after 100 g PEG L^{-1} (left) or 80 mM NaCl (right) treatment and no treatment. Red color indicates up regulation, and blue color indicates down regulation.

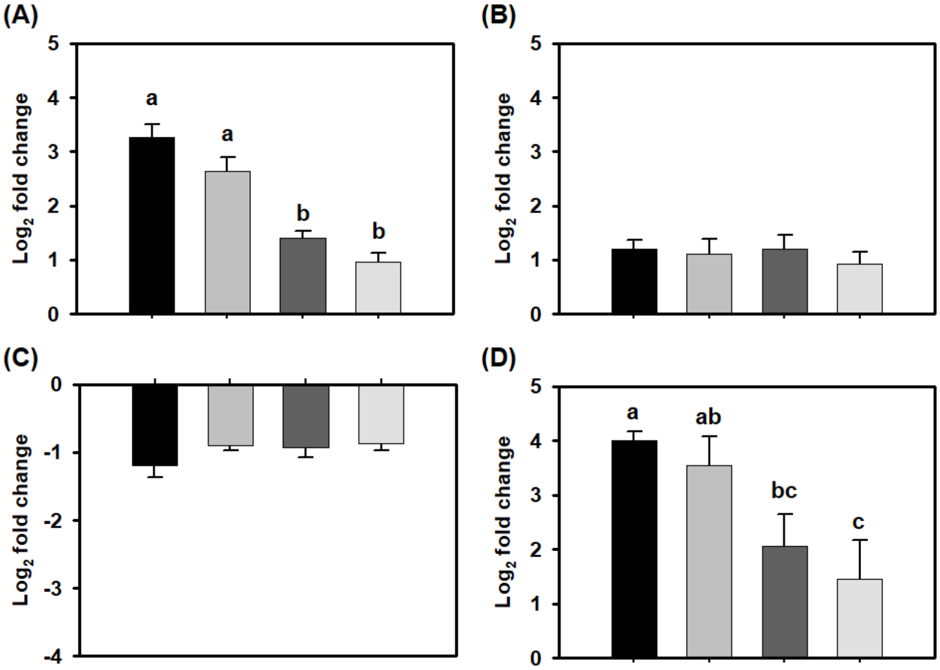
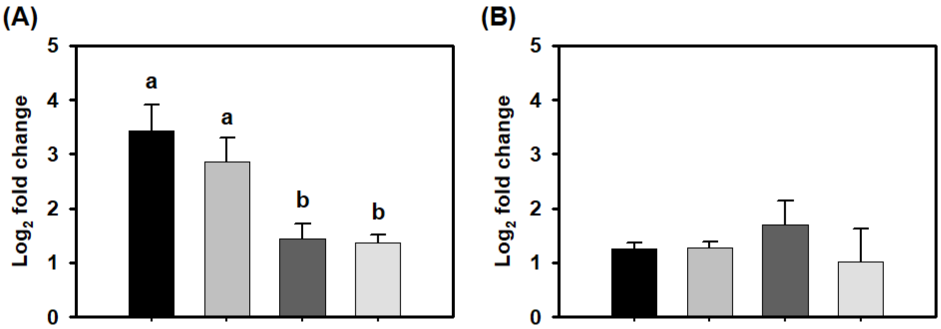


Figure 16. Expression (Log$_2$ fold change between 4 hours after 100 g PEG L^{-1} treatment and no treatment) of genes related to ABA biosynthesis and catabolism; *ZEP* (A), *ADH* (B), *AAO3* (C), and *CYP707A4* (D). The bar graph indicates *E. oryzoides, E. oryzicola, E. crus-galli* var. *praticola,* and *E. colona* from the left. The same letter indicates no significant difference and different letters indicate significant differences at P<0.05 determined by Tukey-HSD.



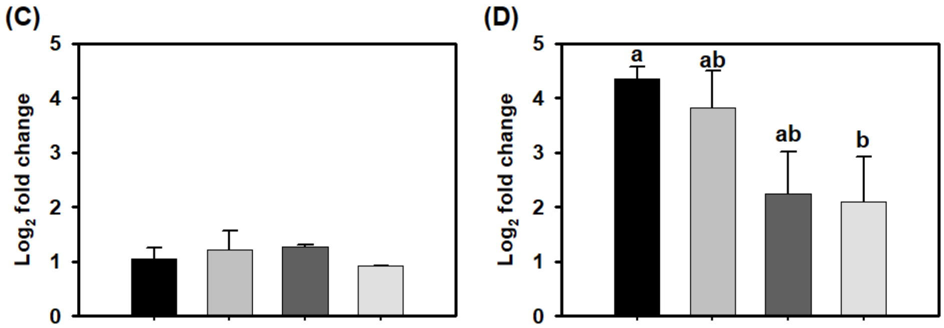


Figure 17. Expression (Log_2 fold change between 4 hours after 80 mM NaCl treatment and no treatment) of genes related to ABA biosynthesis and catabolism; *ZEP* (A), *ADH* (B), *AAO3* (C), and *CYP707A4* (D). The bar graph indicates *E. oryzoides, E. oryzicola, E. crus-galli* var. *praticola,* and *E. colona* from the left. The same letter indicates no significant difference and different letters indicate significant differences at $P<0.05$ determined by Tukey-HSD.

The expression level of *ZEP* (*ABA1*) and *ADH* varies from species to species and is generally known to increase as drought stress increase. In this experiment, the expression level of *ZEP* and *ADH* increased in all four species in both PEG and NaCl treatment. However, the increase pattern was different between two gene expressions. The expression level of *ZEP* was further increased in sensitive species such as *E. oryzoides* and *E. oryzicola*, whereas *ADH* expression level was similarly increased among four species. Moreover, the degree of increase in *ADH* expression was much smaller than that of *ZEP*. On the other hand, *AAO3*, which also encodes a key enzyme in ABA biosynthetic pathway, only increased in the sensitive species in NaCl treatment. When comparing PEG and NaCl treatment, *ZEP* and *ADH* expressions were more increased in NaCl treatment and the *AAO3* expression was increased only in NaCl treatment. This is because 80 mM NaCl treatment induces stronger or faster osmotic stress than 100 g PEG L^{-1} treatment, even if both concentrations are about lethal concentration of the sensitive species.

Besides ABA synthesis, ABA catabolism is also important for controlling endogenous ABA level and *CYP707A* families encode ABA 8′-hydroxylases, which work for a hydroxylation of ABA. Therefore, *CYP707A4* increased significantly in all the *Echinochloa* spp. in both PEG and NaCl treatment, compared to control. However, the amount of RNA expression was more in sensitive species, indicating more catabolism of ABA.

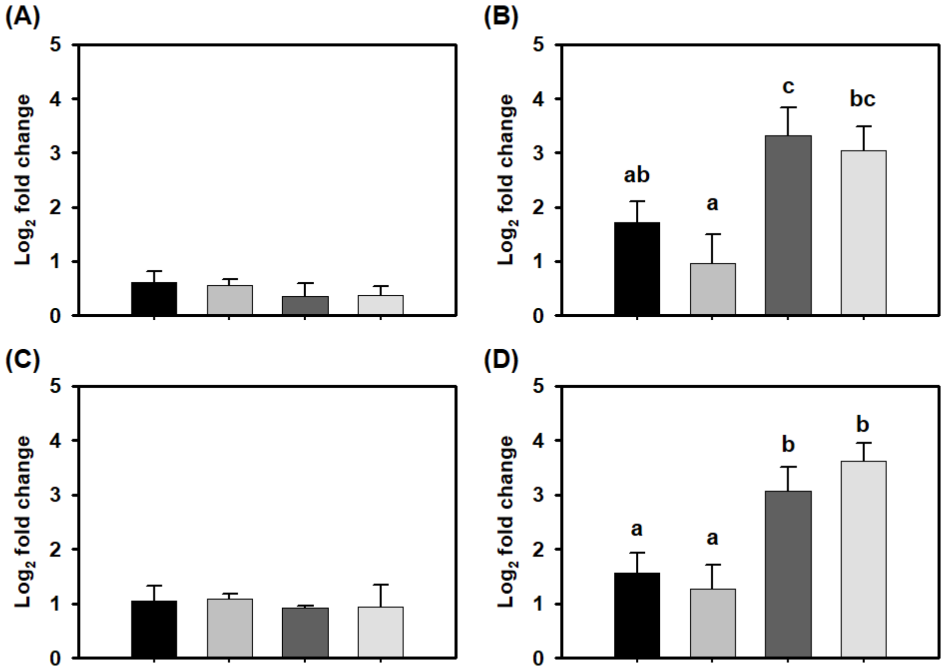
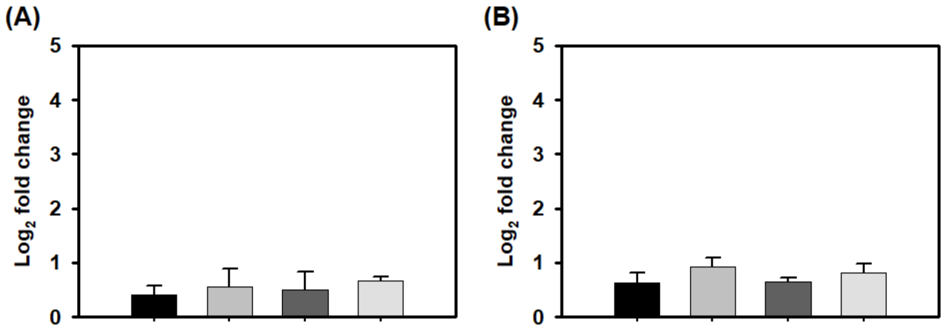


Figure 18. Expression (Log$_2$ fold change between 4 hours after 100 g PEG L^{-1} treatment and no treatment-above two, and 80 mM NaCl treatment and no treatment-below two) of genes related to plant temperature, such as protein kinases involved in stomatal closing and heat shock protein; *NPX1* (A), *HSP70* (B) in PEG treatment, *NPX1* (C), *HSP70* (D) in NaCl treatment. The bar graph indicates *E. oryzoides, E. oryzicola, E. crus-galli* var. *praticola,* and *E. colona* from the left. The same letter

indicates no significant difference and different letters indicate significant differences at $P<0.05$ determined by Tukey-HSD.

Beside the genes directly related to osmotic stress, there are also many protein kinases and transcription factors that regulate stress-inducible gene expression in upstream level. Protein kinases, which are known to function to control opening and closing of stomata, such as *NPX1*, *CPK*s and *MYB15* (Boudsocq and Sheen, 2013; Cominelli et al., 2010; De Leonardis et al., 2012), showed similarly upregulated responses regardless of the species and the kind of treatment.

When stomata are closed, temperature rise is inevitable (Farooq et al., 2009). As temperature rises, plants produce a unique set of proteins called heat shock proteins (HSPs) (Taiz and Zeiger, 2010). Because of different RNA expression level of *HSP70*, the tolerant species, *E. colona* and *E. crus-galli* var. *praticola* were expected to produce much more HSPs than the sensitive species, *E. oryzicola* and *E. oryzoides* in both PEG and NaCl treatment.



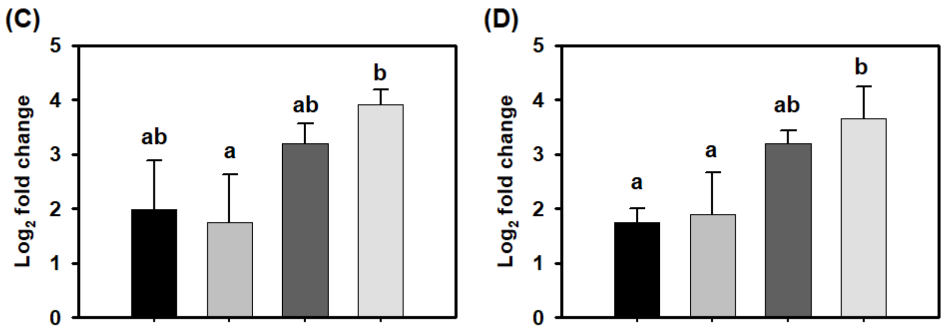


Figure 19. Expression (Log$_2$ fold change between 4 hours after 100 g PEG L^{-1} treatment and no treatment-above two, and 80 mM NaCl treatment and no treatment-below two) of genes related to ion partitioning; *SOS1* (A), *VHA-A1* (B) in PEG treatment, *SOS1* (C), *VHA-A1* (D) in NaCl treatment. The bar graph indicates *E. oryzoides, E. oryzicola, E. crus-galli* var. *praticola,* and *E. colona* from the left. The same letter indicates no significant difference and different letters indicate significant differences at $P<0.05$ determined by Tukey-HSD.

Unlike PEG treatment which cannot penetrate cell membrane, NaCl passes through cell membrane and accumulates in cytosol, divided into Na^+ and Cl^-. Therefore, in order to reduce the harm caused by ion toxicity, it is very important to redistribute ions and to keep ion homeostasis. *SOS1* which encodes Na^+-H^+ antiporter and *VHA-A1* which encodes vacuolar H^+-ATPase A1 are almost twice more expressed in the tolerant species, *E. colona* and *E. crus-galli* var. *praticola*. Moreover, these genes showed the different expression pattern from that of PEG induced osmotic stress in which these genes were expressed to a similar extent to control (0 g PEG L^{-1}) even in the tolerant species.

5. DISCUSSION

During germination test, *E. colona* was the most tolerant to osmotic stress, having the highest GR_{50} value in PEG and NaCl treatment, while *E. oryzicola* (USA) was the most sensitive. This phenomenon is consistent with overall tendency and sensitivity of tolerance. However, *E. crus-galli* var. *praticola*, which is considered tolerant species, and *E. oryzoides*, which is considered sensitive species showed intermediate GR_{50} in PEG and NaCl treatment. Although the high germination rate does not always mean high survival rate or high adaptability, this finding showed the possibility that the germination rate is able to influence the adaptability of the species.

During seedling emergence test, root to shoot (R/S) ratios were measured. R/S ratios in the tolerant species are expected to increase or remain similar as osmotic stress intensity increases (Park et al., 2016). This is because growth of root tends to continue in order to ensure appropriate water intake, compared to shoot which stops growing as soon as exposed to osmotic stress (Taiz and Zeiger, 2010). R/S ratio of *E. oryzoides* decreased as PEG- and NaCl-induced osmotic stress increased. This is because the reduction of root growth was bigger than that of shoot growth, which means sensitiveness to PEG- and NaCl-induced osmotic stress (data not shown). On the other hand, R/S ratios of *E. colona* and *E. crus-galli* var. *praticola* increased as PEG-induced osmotic stress increased. Moreover, similar results were observed to NaCl-induced osmotic stress, when it comes to *E. colona*. However, *E. crus-galli* var. *praticola* showed intermediate response to NaCl treatment, which is similar to that of *E. oryzicola* and *E. crus-galli* var. *crus-galli*. It is thought to be due to its relatively weak adaptability to NaCl-induced saline stress, particularly ion toxicity, during germination and seedling stage. This ability of *E. colona* and *E. crus-galli* var. *praticola* (to only PEG-induced stress) to maintain root growth during osmotic stress is thought to be one of the most important tolerance strategies during water deficit situation, helping water uptake from deeper or further water source. This phenomenon is expected to be related to the adaptability in actual dry habitats of *E. colona* and *E. crus-galli* var. *praticola*.

During early juvenile plant growth test, *E. oryzoides* and *E. oryzicola* began to show leaf curling and wilting at lower PEG and NaCl concentration and showed more leaf curling and wilting at the same PEG and NaCl concentration than *E. colona* and *E. crus-galli* var. *praticola*. This initial physiological response leaded to final difference of GR_{50} value of fresh weight between tolerant and sensitive to osmotic stress.

This visual phenomenon also leaded to more prominent increase of plant temperature in *E. oryzoides* and *E. oryzicola*. This is because the dry parts, which also can be called dead parts, do not have water flow inside, so they can't release heat caused by strong light source (Yang et al., 2013). On the other hand, beside the effects related to the complete necrosis of leaf, plant temperature and chlorophyll fluorescence are also influenced even by slight osmotic stress (Baker, 2008; Kim et al., 2014; Chen at al., 2005). Therefore, plant temperature increased and chlorophyll fluorescence decreased as the stress intensity became stronger in all the *Echinochloa* spp. used in the experiment. However, the degree of change was different between tolerant and sensitive species. For example, in the case of PEG treatment, *E. oryzoides* and *E. oryzicola*, which seem sensitive to osmotic stress, showed significant temperature increase and chlorophyll fluorescence decrease compared to the insignificant change tendency of *E. colona* and *E. crus-galli* var. *praticola* which seem tolerant to osmotic stress.

When plants encounter osmotic stress, stomatal conductance decreases and stomata tend to close in order to reduce water loss through transpiration. Even though stomatal conductance was not measured in this experiment, it can be easily predicted that stomata were more closed as the PEG and NaCl concentration increased, because plant temperature increased according to stress treatment in all the *Echinochloa* spp. However, unlike Zhang's research (Zhang et al., 2006), in which PEG-tolerant rice showed higher temperature increase compared to sensitive rice, *E. colona* and *E. crus-galli* var. *praticola* showed lower temperature increases compared to *E. oryzoides* and *E. oryzicola*. In this experiment, it is unclear whether actual differences in stomatal conductance exist between tolerant and sensitive species at the same PEG and NaCl concentration. However, because protein kinases known to regulate stomatal opening and closing, such as *NPX1*, *CPK*s and *MYB15* (Boudsocq and Sheen, 2013; Cominelli et al., 2010; De Leonardis et al., 2012) showed similarly increased expression regardless of the kind of species, it is carefully inferred that there is no big difference of stomatal opening and closing among species.

It also became clear through qRT-PCR that heat shock proteins of *E. colona* and *E. crus-galli* var. *praticola* contributed to their tolerance to heat stress, which is indirectly induced by stomatal closure, caused by osmotic stress. The difference of *HSP70* expression level, which is known to repair and protect cellular proteins damaged by stress (Franzellitti and Fabbri, 2005), supported it. When stomata are closed, temperature rise is inevitable (Farooq et al., 2009). Therefore, how to manage heat or heat-related damage inside plants can be a key factor for osmotic stress resistance.

Moreover, abscisic acid (ABA) which regulates plant growth and stomatal aperture can also be an important factor, when considering the adaptability of osmotic stress. In this experiment, the expression of genes which encode enzymes involved in biosynthesis and catabolism of ABA is considered through qRT-PCR, instead of directly measuring the amount of endogenous ABA. The gene expression levels included in ABA biosynthesis, such as *ZEP* and *ADH* (Barrero et al., 2006; Finkelstein, 2013; Hadiarto and Tran, 2011) increased in all four species in both PEG and NaCl treatment, meaning the increase of ABA production, compared to no treatment. In particular, the expression level of *ZEP* was further increased in sensitive species, suggesting that the species susceptible to osmotic stress such as *E. oryzoides* and *E. oryzicola* are hypersensitive to the same stress, which well-adapted species regard as minor or tolerable stress. Moreover, *CYP707A4*, which is involved in ABA catabolism (Kushiro et al, 2004; Saito et al., 2004; Shinozaki and Yamaguchi-Shinozaki, 2007; Verslues, 2016), increased significantly in all the *Echinochloa* spp. in both PEG and NaCl treatments, compared to control. However, the amount of gene expression was bigger in sensitive species, meaning more ABA catabolism. In conclusion, it can be inferred that much more ABA was synthesized and much more ABA was removed in the sensitive species, such as *E. oryzoides* and *E. oryzicola*, consuming internal energy. It can be a reason why *E. oryzoides* and *E. oryzicola* are more sensitive to osmotic stress than *E. colona* and *E. crus-galli* var. *praticola* and this kind of inefficiency would make it hard for *E. oryzoides* and *E. oryzicola* to adapt to dry land.

In the case of NaCl treatment, in addition to osmotic effects caused by low water potential gradient, specific ion toxicity effects, in particular Na^+ and Cl^-, also occur. Therefore, when the ions penetrate into the cell membrane, it is important to exclude and compartment these ions in the cytosol (Taiz and Zeiger, 2010; Zhu, 2007). *E. colona* and *E. crus-galli* var. *praticola* showed more increased *SOS1* expression level than *E. oryzicola* and *E. oryzoides*, in NaCl treatment, which encodes plasma membrane Na^+-H^+ antiporter. Through *SOS1*, *E. colona* and *E. crus-galli* var. *praticola* are expected to be able to antagonize the ionic influx caused by NaCl treatment. Furthermore, overexpression of *SOS1* in transgenic plants are known to enhance salt tolerance (Shi et al., 2003). In addition to *SOS1*, the expression of *VHA-A1*, which encodes vacuolar-H^+-ATPase component, also supported it.

In conclusion, *E. colona* showed the greatest tolerance against PEG- and NaCl-induced osmotic stress, followed by *E. crus-galli* var. *praticola*, confirming that they are the best-adapted species to dry land condition. In contrast, *E. oryzicola* and *E. oryzoides* showed relatively weak adaptability to osmotic stress, explaining why these *Echinochloa* species cannot be found in dry upland area. This phenomenon is consistently observed during germination, seedling emergence, and early vegetative stage, even though *E. curs-galli* var. *praticola* showed intermediate characteristics against NaCl-induced osmotic stress during germination and seedling emergence stage.

It is expected that *Echinochloa crus-galli*, hexaploid and mostly widely distributed species, is evolved by natural hybridization between tetraploid *E. oryzicola* and unknown diploid *Echinochloa* species and chromosome duplication (Yabuno, 1983). *E. oryzicola* inhabits paddy field but not upland field, while *E. crus-galli* inhabits a broad range of habitats from paddy field to upland field depending on variety and ecotype (Yamasue et al., 1989b). It suggests that tetraploid, which is adapted to paddy field, evolved into hexaploid with various habitats, while hybridizing with unknown diploids, which have more various habitats.

Through this experiment, it was confirmed that there exist differences in water availability of each *Echinochloa* spp. and this water availability can explain their actual habitats of *Echinochloa* species. *E. colona* and *E. crus-galli* var. *praticola*, which showed the greatest tolerance against osmotic stress, inhabit dry land condition, whereas *E. oryzicola* and *E. oryzoides* which showed relatively weak adaptability to osmotic stress, inhabit flooded paddy field. That is, *Echinochloa* spp. have differently adapted to the environment, particularly in accordance with water availability.

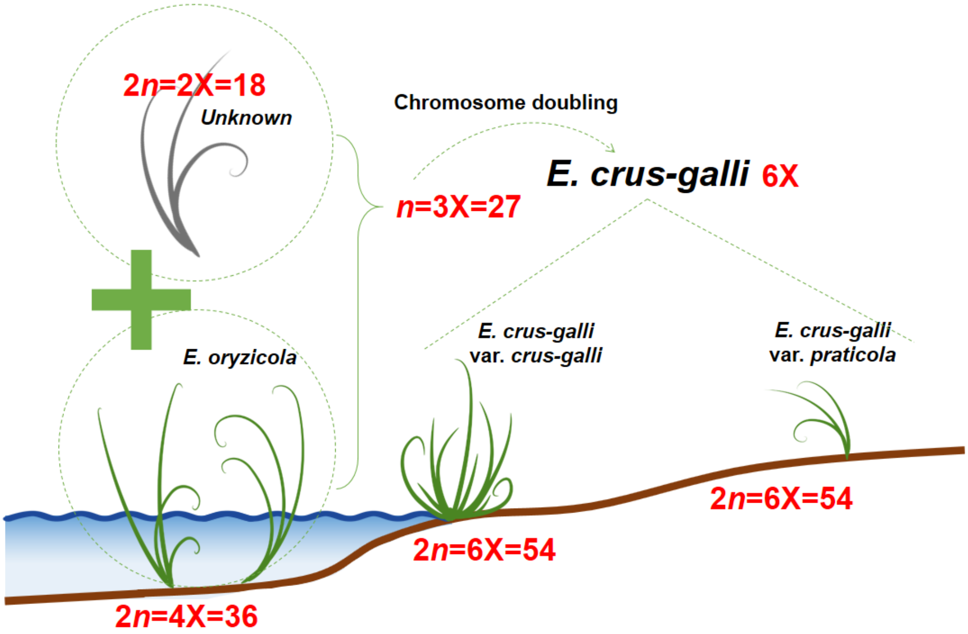


Figure 20. Summary of evolutionary ecological adaptation in *Echinochloa* species (based on Yabuno's hypothesis (Yabuno, 1983) and our findings).

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국 문 초 록

피 속 종들의 건조 적응성에 관한 생리적, 분자생물학적 고찰

유혜진

작물생명과학전공

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피 속(*Echinochloa* spp.)에는 약 50여종이 속해있다고 추정되며, 대표적으로 국내에서는 이들 중 강피(*Echinochloa oryzicola*), 물피(*E. crus-galli* var. *crus-galli*), 돌피(*E. crus-galli* var. *praticola*)가 주로 분포하는 것으로 알려져 있다. 하지만 이들이 주로 분포하는 서식지를 살펴보면, 강피는 담수 조건인 논에서만, 물피는 논과 논둑에서, 돌피는 밭에서만 발견된다는 점에서 특이점을 가진다. 이를 바탕으로 본 연구는 피 속 식물 종들의 서식지의 차이가 수분 스트레스에 대한 적응력과 관련이 있을 것으로 추정하고 이를 확인하는 것을 목표로 진행되었다. 건조 스트레스를 유발하기 위해서 PEG (polyethylene glycol)와 NaCl을 사용하였고, 식물의 반응은 발아(petri-dish법), 유묘생장(growth pouch법), 초기영양생장(pot법)을 포함한 총 3가지 단계에서 측정하였다. 발아단계에서는 발아를 50% 억제하는 PEG와 NaCl 농도인 GR_{50}

값에서, 유묘생장단계에서는 지하부와 지상부의 생육 비율인 R/S 값에서, 초기 영양 생장 단계에서는 생체중, 식물 체온, 엽록소 형광 반응 등에서 건조 적응성이 높은 *E. colona*, 돌피(*E. crus-galli* var. *praticola*)와 상대적으로 건조 적응성이 낮은 *E. oryzoides*, 강피(*E. oryzicola*)의 차이를 확인할 수 있었다. 또한 이를 유전자 발현 차이로 해석하고자, qRT-PCR을 이용하여 건조 스트레스에 대한 적응성 및 내성과 관련된 유전자들의 발현량 차이를 비교해보았다. 그 결과 건조 스트레스 상황에서 건조 적응성이 높은 *E. colona*, 돌피(*E. crus-galli* var. *praticola*)와 건조 적응성이 낮은 *E. oryzoides*, 강피(*E. oryzicola*)에서 ABA 합성 경로와 SOS 경로에 관여하는 유전자 발현량의 차이를 확인할 수 있었다. 이러한 건조 스트레스에 대한 식물체의 반응 차이는 피 속 종들이 실제로 서로 다른 서식지에 적응하고, 분포하는 현상을 잘 설명할 수 있을 것이라고 생각된다.

주요어 : 건조 적응성, 피, 수분 스트레스, 서식지 다양성

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